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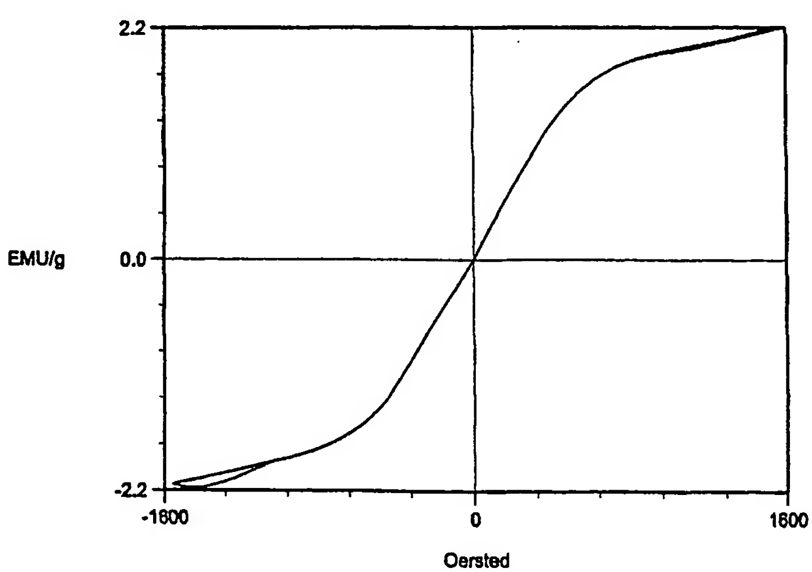

(57) Abstract

Stable biocompatible polymeric microspheres include a polymer shell encapsulating a superparamagnetic magnetite core. The microspheres are prepared by the controlled synthesis of magnetite and encapsulating the magnetite with a polymer coat in a unitary process within the confines of a water droplet of a water-in-oil microemulsion. The microemulsion water droplet acts as a microreactor and enables the control of the size of the magnetite core and the polymer coating. The resulting polymeric microspheres, as synthesized, have a narrow size distribution and each contains a single stabilized superparamagnetic magnetite crystal core encapsulated within a coat of a biocompatible random co-polymer that is capable of being functionalized.

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CONTROLLED SIZE POLYMERIC MICROSPHERES WITH SUPERPARAMAGNETIC CORES

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BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to controlled size polymeric microspheres with superparamagnetic cores. More specifically the invention relates to a unitary process for preparing superparamagnetic magnetite particles encapsulated by homopolymers or copolymers using a water-in-oil microemulsion system. The same process can be used to synthesize polymeric microspheres with a ferro- or ferrimagnetic core. The process of the invention synthesizes both the magnetite core and the polymeric shell in a continuous process within the microdroplets of a water-in-oil microemulsion. Stable, biologically compatible, polymeric microspheres of controlled and uniform size distribution containing superparamagnetic cores can be prepared using the process of the invention.

2. Description of the Related Art

Polymeric microspheres containing magnetic particles are used in biological and medical research as well as in therapy. They are used as carriers of pharmaceutically active substances which can be targeted to a particular site by an external magnetic field and have been particularly useful in cancer therapy for biophysical targeting of anti-tumor agents.

Magnetizable polymeric microspheres are also used as contrast agents for nuclear magnetic resonance (NMR) imaging. (See, e.g., S. Frank, P.C. Lauterbur, *Nature 363*, 334-336 (1993); E.C.Wiener, et al., *Magn. Reson. Med. 31*, 1-8 (1994)).

Magnetic resonance imaging (MRI) for medical diagnosis utilizes contrast agents to improve the contrast between normal or background tisssue and diseased or target tissue by altering molecular parameters such that image enhancement is achieved. The general requirements for clinically useful magnetic resonance contrast agents are magnetic activity

which alters image signal intensity, biodistribution to target or normal tissue, exclusion by background or diseased tissue, and low toxicity. Superparamagnetic materials are especially useful as contrast agents because they have large effects on NMR images.

Magnetic particles have different types of magnetic behavior depending on their size and the conditions of their preparation. These particles can be ferromagnetic, paramagnetic or superparamagnetic.

Ferromagnetism occurs when the unpaired electrons in the magnetic material are highly coupled leading to a high degree of magnetic alignment. This interaction of the unpaired electrons leads to ferromagnetic materials having particular characteristics. Ferromagnetic materials have high magnetic susceptibilities, meaning that they are very responsive to an applied magnetic field. They also retain their magnetic properties after the magnetic field has been removed.

Paramagnetic substances contain unpaired electrons that are not coupled. Their unpaired electrons do not interact. Paramagnetic substances have weak magnetic susceptibilities, and become weakly magnetic in the presence of a magnetic field but rapidly demagnetize when this field is removed.

Superparamagnetic substances have characteristics of both ferromagnetic and paramagnetic substances. They have high magnetic susceptibilities but demagnetize rapidly once the magnetic field is removed. A substance that is superparamagnetic is of a size such that it has a single magnetic domain. Therefore, superparamagnetic substances do not remain magnetic in the absence of an applied magnetic field.

Colloidal iron oxide particles, such as magnetite (Fe_3O_4) or gamma ferric oxide (Fe_2O_3) are superparamagnetic when the crystals of iron oxide are sufficiently small, e.g. less than about 300-500 Å in size. Biocompatibility can be achieved by coating the particles with a biocompatible matrix to form microspheres. The polymer matrix should be chemically inert in biological systems and should possess a diameter much below 1 μm, i.e., of the order of about 100 nm, in order to enable the microspheres to circulate once injected within the vascular system. See Widder, K.J. et al., *Advances in Pharmacology and Chemotherapy 16*, 213-271; and Ring, G.C. et al., *Am. J. Physiol. 200* (1961) 1191. Such microspheres are particularly useful as magnetic resonance contrast agents.

Polymeric microspheres containing a core of iron oxide have been synthesized in two-

step systems. In a first step, iron oxide particles are made by bulk precipitation. In bulk precipitation of iron oxide particles, an aqueous solution of metal salts is prepared and base is added for particle precipitation. Surfactant is sometimes added to prevent aggregation and generally the precipitate is ground or ball-milled to the desired particle size. The iron oxide particles are then recovered, commonly by magnetic separation. In a second step, the recovered particles are coated with a biocompatible material.

Two-step methods of this type for preparing microspheres are described, for example, in U.S. Patent Nos. 4,206,094 and 4,219,411 to Yen et al., 4,454,234 to Czerlinski, 4,863,715 to Jacobsen et al., 4,965,007 to Yudelson, 5,512,268 to Grinstaff et al., 5,356,713 to Charmot et al., 5,597,531 to Liberte et al., and WO 90/01295.

U.S. Patent Nos. 4,206,094 and 4,219,411 to Yen et al. describe microspheres with finely divided iron oxide dispersed throughout. The microspheres are produced by aqueous suspension polymerization or aqueous emulsion polymerization of water soluble acrylic monomers in the presence of commercially available metal oxide particles.

U.S. Patent No. 4,454,234 to Czerlinski describes coated magnetizable particles having a ferro- or ferrimagnetic core of metal oxide surrounded by an acrylic polymer. The core material is first ground or pulverized, e.g. by ball milling, and the particles are homogeneously distributed in a liquid phase of monomers which are then polymerized around the particles. The coating is at a temperature below the Curie temperature of the metal oxide so that the particles do not aggregate. The metal oxide cores are not made *in situ* and they are not superparamagnetic.

U.S. Patent No. 4,863,715 to Jacobsen et al. describes ferromagnetic particles homogenized by sonication and/or shaking and coated with polymers such as a cellulose derivative by immersing the particles in a solution containing the polymer.

U.S. Patent No. 4,965,007 to Yudelson describes the preparation of superparamagnetic magnetite particles by mixing ferric and ferrous salts, water and acid and then adding base to precipitate the particles. The particles are recovered by magnetic separation, washed with water, and encapsulated by a cross-linked coacervate of gelatin and hydrophilic polymer.

U.S. Patent No. 5,512,268 to Grinstaff discloses encapsulation of small superparamagnetic particles such as iron oxide by first dispersing the particles in a medium

such as a fluorocarbon or soybean oil and then entrapping them in a polymeric shell of sulfhydryl-containing polymer which is cross-linked by way of disulfide bonds.

U.S. Patent No. 5,356,713 to Charmot et al. describes first preparing magnetizable particles of less than 30 nm by a sol gel method. This is followed by flocculation and separation of the particles and then dispersing the particles in an organic medium. A shell of hydrophobic crosslinked copolymer is polymerized around the particles.

U.S. Patent No. 5,597,531 to Liberti et al. describes the preparation of coated superparamagnetic particles. Magnetite is first prepared by base precipitation of ferric and ferrous salts in solution and aggregates of magnetite are disrupted, e.g. by sonication, in the presence of coating material such as dextran, proteins, polypeptides, polymers, copolymers and detergents.

WO 90/01295 to Menz et al. describes the preparation of superparamagnetic metal oxides, particularly iron oxide, associated with ligands. The metal oxides are precipitated from the metal salts in the presence of the ligands. The metal oxide precipitation follows the prior art methods of precipitation from aqueous solution by addition of base to form particles of non-uniform size and the products are subjected to ultrafiltration. The ligands are macromolecular species including serum proteins, hormones, asialoglycoproteins, galactose-terminal species, polysaccharides arabinogalactan or conjugates of these with poly(organosilane) or dextran.

Other types of iron oxide-containing microspheres are described, for example, in U.S. Patent Nos. 4,157,323 to Yen et al., 5,071,076 to Chagnon et al., 5,206,159 to Cohen et al., 5,219,554 to Groman et al., and 5,427,767 to Kresse et al.

U.S. Patent No. 4,157,323 to Yen et al. describes microspheres produced by *in situ* addition polymerization of an aqueous monomer mixture which also contains metal particles or particles of magnetic iron oxide. Polymerization conditions must be such that oil droplets, as in emulsion polymerization, do not form.

U.S. Patent No. 5,071,076 to Chagnon et al. describes controlled size magnetic microparticles produced by milling slurries of metallocene and metal hydroxide. Particle size is controlled by milling the particles. The particles are uncoated.

U.S. Patent No. 5,206,159 to Cohen et al. describes polymer particles of a non-ionic cross-linked polyacrylamide gel with dispersed superparamagnetic particles. The polymer

particles are swollen in a solution of iron salt such that the iron salt is taken up by the polymer particles. The iron salt is then converted, *in situ,* to superparamagnetic iron oxide colloidally dispersed within the polymer matrices. There is no single metal oxide core nor is there any suggestion of *in situ* polymerization.

U.S. Patent No. 5,219,554 to Groman et al. describes superparamagnetic fluids containing metal oxide particles, particularly iron oxides, which may be surrounded by a coating of polysaccharide, protein, polypeptide or organosilane. The metal oxide particles are prepared from metal salts by base precipitation. The precipitation can occur in the presence of the coating material to form the coated particles. The metal oxide can also be oxidized to form a soluble metal oxyhydroxide. There is no suggestion of a polymeric microsphere formed with *in situ* polymerization.

U.S. Patent No. 5,427,767 to Kresse et al. describes the preparation of nanocrystalline magnetic particles having an iron oxide core with a glycosaminoglycan envelope such as chondroitin chemisorbed to the core. The iron oxide is precipitated from aqueous solution by the addition of base. The precipitation is in the presence of the coating agent.

Kawaguchi, H. et al., *Colloids and Surfaces A: Physicochem. Eng. Aspects 109*, 147-154 (1996) prepare polymeric microspheres containing magnetite by swelling dried polymeric microsphere with an aqueous solution of Fe^{2+} and Fe^{3+} followed by base precipitation using ammonia to form magnetite. These microspheres have magnetite particles distributed throughout, but located mainly at the surface of the microsphere.

Other processes for preparing iron oxide particles have used microemulsions, but have not suggested the use of the microemulsion microdroplets to prepare controlled size polymeric microspheres with a magnetite core.

Gao, M. et al., *Thin Solid Films 248*, 106-109 (1994) prepare a composite microgel of ultrafine Fe_2O_3 particles covered with a methylacrylic acid-divinylbenzene copolymer. This microgel is prepared by forming the Fe_3O_2 particles in a toluene- water solution in the presence of methacrylic acid as a surfactant, the resulting particulate organosol of methacrylate covered Fe_2O_3 is then mixed with divinylbenzene and crosslinked to form the composite microgel. There is no suggestion of the use of microemulsion water droplets to control the size and character of the coated particles.

Liz, L. et al., *Journal of Material Science 29*, 3797-3803 (1994), describe the

preparation of colloidal magnetite (Fe_3O_4) ultrafine particles in the droplets of a microemulsion system. They indicate that the size of the magnetite particles can be controlled by controlling the size of the microdroplets in the microemulsion. There is no suggestion of coating the magnetite particle with a polymer within the confines of the same microdroplet in the microemulsion.

Candau, F. et al., *Journal of Colloid and Interface Science 101*, 167-183 (1984) prepare polyacrylamide particles in a water-in-oil microemulsion.

A process for preparing biocompatible polymeric microspheres of controlled and uniform size with superparamagnetic magnetite particle cores is still needed, especially to provide biocompatible superparamagnetic microspheres for use as MRI contrast agents. It is an object of the invention to provide an economical and efficient method for providing biocompatible microspheres (with a superparamagnetic core) of controlled and uniform size.

SUMMARY OF THE INVENTION

These and other objectives are achieved by the present invention, which provides a process for preparing controlled size polymeric microspheres containing a superparamagnetic (or ferro-/ferrimagnetic) core. The polymeric microspheres prepared contain single cores of stable magnetite. The process of the invention allows for the close control of both the size of the core as well as the thickness and the porosity of the polymer coating. Although a preferred embodiment of the invention prepares microspheres containing a superparamagnetic core, the process of the invention can also be used to prepare polymeric microspheres that contain a ferromagnetic or a ferrimagnetic core.

A preferred embodiment of the process of the invention prepares polymeric microspheres containing a single particle, monocrystalline core of superparamagnetic magnetite. The magnetite core is stabilized by a dispersing agent. This core is encapsulated by a polymer coating that is preferably biocompatible and functionalizable. The term "functionalized," means that the coating has reactive groups which are suitable for covalent coupling to molecules that will impart additional useful characteristics to the microspheres such as specific localization and visibility. The preferred polymer coating is functionalizable due to the presence of carboxyl and/or hydroxyl groups. These groups are amenable to the attachment of probes or markers, medical drugs, and antibodies by chemical reactions.

One embodiment of the invention prepares superparamagnetic polymeric microspheres in a unitary (one-pot) process using a water-in-oil microemulsion. The water-in-oil microemulsion is prepared by first combining an aqueous solution of salts of Fe (II) and Fe (III), oil, polymerization initiator, polymer monomers, and a surfactant that locates at the oil-water interface. A water-in-oil microemulsion has been formed. Then base and a dispersing agent which is water soluble and locates in the water phase are added. The microemulsion is then mixed to precipitate magnetite within the microemulsion microdroplets. In a preferred embodiment of the invention, the magnetite precipitated is in the form of single particle superparamagnetic crystals which are stabilized by the dispersing agent. Energy is then applied to the magnetite containing water-in-oil microemulsion to initiate the polymerization of the monomers and formation of a polymer coating around the magnetite crystals. Polymeric microspheres containing a core of a single stabilized magnetite particle are thus formed. Preferably a polymer crosslinker is added to the microemulsion before polymerization. The whole process of formation of the polymeric microsphere takes place within the confines of the microdroplets in the microemulsion.

The addition of base and a dispersing agent may be added via an aqueous solution or another microemulsion containing base and dispersing agent within its microdroplets.

A preferred surfactant that locates at the oil-water interface is AOT (sodium-bis-2-ethyl-hexyl sulfosuccinate).

Preferred oil phases are toluene and cyclohexane.

A preferred dispersing agent is tetramethylammonium hydroxide (TMAOH).

Preferred bases for the process of the invention include KOH, NaOH, LiOH, and NH_4OH. A more preferred base is tetramethylammonium hydroxide (TMAOH).

Preferably TMAOH is used both as the base and as the dispersing agent.

The TMAOH solution is preferably an aqueous solution of from about 1% to about 25% TMAOH. More preferably the TMAOH solution is an aqueous solution of about 5-10% TMAOH.

The mixing of the microemulsion to precipitate magnetite is preferably carried out by sonication.

Preferably the stoichiometric molar ratio of Fe (III) to Fe (II) is in the range of about 1 to about 3, more preferably this ratio is about 2.

Water soluble Fe salts can be used in the process of the invention. Among water soluble Fe salts, preferred ones are halides, sulfates, acetates, and nitrates. More preferred Fe salts are chlorides.

A preferred water-in-oil microemulsion for the process of the invention is formed from AOT, toluene, and water.

The process of the invention can vary the weight percent of AOT, toluene, and water in forming AOT/toluene/water microemulsions.

Preferably the ratio of AOT to water is from about 2 to about 20. More preferably the ratio of AOT to water is from about 5 to about 11.

The initiators that can be used in the process of the invention include both hydrophobic initiators and hydrophilic initiators. A preferred initiator is the hydrophilic initiator $K_2S_2O_8$ (potassium persulfate). A more preferred initiator is the hydrophobic initiator AIBN (2,2'-azobisisobutyronitrile).

Preferred monomers for the process of the invention should be biocompatible and nontoxic. Methacrylic acid and hydroxyethyl methacrylate are preferred monomers. Preferably the ratio of methacrylic acid to hydroxyethyl methacrylate is from 100% methacrylic acid to about 0.1% methacrylic acid.

The energy source for initiating the polymerization can be either heat or radiation. Preferably the step of applying energy is carried out by heating in the range of from about 30°C to about 80°C. More preferably, the range of from about 45°C to about 65°C can be used for this step of the process of the invention.

A preferred crosslinker for the process of the invention is N,N'-methylenebisacrylamide. A more preferred crosslinker is pentaerythritol triacrylate.

The process of the invention has a number of significant advantages. For example, the process allows for control of the size of both the core and the polymeric shell by preparing both the core and the polymeric coating in a continuous process within the microdroplets of a microemulsion. By synthesizing magnetite particles and a polymeric shell in a continuous process within the water pool of water-in-oil microemulsions, microspheres of controlled size having superparamagnetic cores have been prepared. Using the process of the invention, microspheres of more uniform size than previously obtainable have been prepared.

Advantageously, the process produces superparamagnetic microspheres having

uniform size cores, e.g., the superparamagnetic polymeric microspheres have a core of a single particle of superparamagnetic magnetite with a radius in the range of 5-10 nm, a saturation magnetization of about 2.7 emu/gram, and a narrow size distribution of the magnetite particles. The polymeric shells can be prepared to have a uniform hydrogel (polymer coat) thickness which can be tailored, in any particular batch, to anywhere between about 30 nm and micron sizes by varying the amount and the nature of components of the microemulsion. The hydrogel has a narrow size distribution with variance on the order of 0.02.

The method takes advantage of the cage-like effect of the water-in-oil droplets to control the size of the magnetite. This produces magnetite particles that are dramatically smaller and better monodispersed in size than any superparamagnetic polymeric microsphere cores previously prepared. By varying the quantities and nature of components of the microemulsion, the concentration of reactants, the ionic strength, and the temperature, a great deal of control can be exercised over the size of the iron oxide core and the polymer coating. The process of the invention can prepare polymeric microspheres of uniform size distribution, tailor made to the specifications needed, e.g. for use as magnetic contrast agents.

Another advantage of the process of the invention is that it comprises an efficient method whereby components are added and processed in a continuous manner, making the process amenable to automation and large scale production, e.g., using laboratory robotics.

These and other advantages of the present invention will be appreciated from the detailed description and examples which are set forth herein. The detailed description and examples enhance the understanding of the invention, but are not intended to limit the scope of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a curve of the magnetization versus the applied magnetic field, as discussed in EXAMPLE 10.

Figure 2 shows an Atomic Force Microscopy (AFM) picture for the microspheres prepared by the process of the invention, as is discussed in EXAMPLE 11.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a process for preparing controlled size polymeric microspheres that have a single stable superparamagnetic core. narrow core as well as shell size distributions, and a variable core/shell size ratio. The process of the invention accomplishes this by synthesizing both the magnetite particle and the polymer coating within the same controlled size reaction medium, i.e. the microdroplet. A preferred embodiment of the invention synthesizes microspheres, comprised of a superparamagnetic magnetite particle with a polymer coating, by controlled precipitation of ferrous and ferric ions within water droplets (also called microdroplets) of a water-in-oil microemulsion followed by polymerization, within the same water droplet, of a biocompatible homopolymer or copolymer (hydrogel).

The invention uses a water-in-oil microemulsion. Microemulsions are optically isotropic, spontaneously formed, and thermodynamically stable dispersions of at least three components. Two immiscible substances, usually "oil" and "water," and a non-ionic or ionic surface active agent, or surfactant. The term "oil" means a nonpolar hydrophobic liquid, and the term "water" means a polar hydrophilic liquid that is not limited to water and includes aqueous liquids and solutions. A water-in-oil microemulsion has a greater proportion of oil than water such that the bulk of the microemulsion or the dispersing medium is oil. For example, a weight percentage of oil of about 70 to 80 % and water of about 2 to 10 % is common, with the surfactant present as another component. These percentages are only illustrative and should not be taken to limit the possible water-in-oil microemulsions that can be prepared.

In a water-in-oil microemulsion, water "microdroplets," or "pools" surrounded by surfactant are formed. These droplets are called the "dispersed" phase and the oil is described as the "continuous" phase or the dispersing medium. The continuous phase refers to the component of the microemulsion present in higher amounts. Microdroplets are stabilized by surfactant and are distributed throughout the continuous phase.

Both non-ionic and ionic surfactants can be used in the preparation of microemulsions. Surfactants are amphiphilic molecules. Amphiphilic molecules have a polar part and an apolar part, and by acting at the interface of the two immiscible substances in a microemulsion, they solubilize the oil and water phases by reducing the surface tension

between them. In a water-in-oil microemulsion, the surfactant molecules reside at the oil/water interface and stabilize the water droplets.

The surfactant that is a component of the microemulsion and stabilizes the microdroplets will be referred to as "surfactant" to distinguish it from the dispersing agent that stabilizes the magnetite particles in solution. The dispersing agent that stabilizes the magnetite particles in solution will be referred to as "dispersing agent."

Water-in-oil microemulsions have uniform sized water droplets in the nanometer size range. The uniform and small size of these droplets makes them an excellent reaction vessel for forming a polymeric microsphere containing a magnetizable core in one continuous reaction. The process of the invention, taking advantage of the properties of microemulsion droplets, uses them as microreactors. It is noted that larger size microdroplets can also be formed. Ionic or hydrophilic reactants are confined to the water droplets, such that reactions with reactants that have polar or ionic character are confined to the water droplets.

The invention uses the cage-like effect and uniform size of the droplets of the microemulsion to create an ideal reaction vessel to control the size of the magnetite particle and the resulting microsphere. This allows for both the preparation of magnetite particles that are small and monodispersed in size and the preparation of controlled size superparamagnetic polymeric microspheres. The controlled precipitation of magnetite in microemulsion followed by the polymerization of hydrogel improves the qualities of the microspheres over the prior systems. The reduced size of the magnetite particles produces superparamagnetism. Also, by carrying out the polymerization reaction in the same medium continuously after core formation, the overall magnetite content of the microspheres is increased as explained below. The process of the invention for the first time precipitates magnetite in a microemulsion followed by the polymerization of a hydrogel coating within the same microdroplet in one continuous reaction.

A preferred microemulsion system for the process of the invention is a water/toluene/AOT water-in-oil microemulsion. AOT, or Aerosol-OT (sodium bis-2-ethyl-hexyl sulfosuccinate), is a preferred surfactant of the water-in-oil microemulsion system used in the process of the invention. AOT is an anionic surfactant which makes the stabilizing layer around the droplet. Other surfactants that locate at the oil water interface can also be used. Preferred surfactants are double or single hydrocarbon chain ionic or non-ionic

surfactants (AOT, Sorbitan alkylates- Span®, hexadecyltrimethylammonium bromide, sodium dodecylsulfate), and polyoxyalkylethylene (hexaoxyethylene) with or without hydrocarbon alcohol as cosurfactant. A more preferred surfactant is AOT without cosurfactant.

Preferred continuous, or oil phases, for the process of the invention are saturated linear or cyclo-hydrocarbons and aromatic hydrocarbons. More preferred oil phases are toluene or cyclohexane.

The thickness of the polymeric shell and the size of the magnetite particle can be controlled by the size of the water droplets, concentration of the reactants, rate of mixing, nature of the surfactant, the dispersing agent, the counter ions, the base, and the temperature. By varying the ratio of the components of the microemulsion, the size of the water droplets (microdroplets) can be controlled. For example, the radius of the water microdroplets can be changed by varying the ratio between water and surfactant. The size of the microdroplets controls the size and character of the magnetite particles and the polymeric (hydrogel) coating. By varying parameters of the microemulsion system as well as the concentration and nature of the reactants, polymeric microspheres can be prepared that are closely tailored to the needs of the user.

For the reactions, a microemulsion of AOT/toluene/water that varies the components such that AOT in an amount from about 10 weight percent to about 30 weight percent, water in an amount from about 2 weight percent to about 10 weight percent, and toluene in an amount from about 70 weight percent to about 80 weight percent based on 100 % AOT/toluene/water mixture can be used. A preferred microemulsion can be prepared using AOT of from about 19 weight percent to about 21 weight percent, water from about 5 weight percent to about 9 weight percent, and the toluene from about 72 weight percent to about 76 weight percent. By selecting different weight percentages for the different components of the water-in-oil microemulsion used in the process of the invention, the size of the microdroplet can be controlled. This then controls the thickness of the polymeric coating and overall size of the microsphere. Choosing weight percentages for each of the components from the ranges indicated above allows for the preparation of polymeric microspheres of a range of desirable sizes.

The mole ratio of AOT to water in the microemulsions can also be used to control the

size of the water droplet and therefore the size of the resulting microsphere prepared using the process of the invention. Mole ratios of AOT to water can be varied from between about 2 to about 12. The ratio of AOT to water is preferably from about 5 to about 11.

The size of the polymer coating can be described as the "thickness" of the coating. The "thickness" of the coating is intended to refer to the radial distance from the surface of the magnetite core to the outer surface of the polymeric microsphere. The thickness of the polymeric coating of the microspheres prepared by the process of the invention can be determined by subtracting the radius of the core from the radius of the polymeric microspheres. These radii can be determined by dynamic light scattering, as described in the examples.

Control of other reaction components such as the concentration of metal ions, monomers used in the polymerization process, and initiators can also control the quality and size of the resulting microsphere. The uniform size of the droplets allow the process of the invention to prepare a uniform population of magnetite cores and polymeric coats. Therefore, the process of the invention prepares superparamagnetic polymeric microspheres of a high quality and uniformity without the need for further purification or separation of microspheres. The microspheres are simply extracted from the microdroplets by washing with organic solvents such as acetone and ethanol.

The core of the polymeric microsphere prepared by the process of the invention is a polymer-shell stabilized particle of magnetite. Magnetite is a mixed iron (II)-iron (III) oxide of the formula Fe_3O_4. It is a black crystal that is strongly magnetic and possessed of a fairly high electrical conductivity. Magnetite exhibits ferrimagnetic behavior, but when magnetite particles are of a small size, they are superparamagnetic.

A variety of approaches are available for the synthesis of magnetite leading to magnetite particles of different shapes and properties. Their synthesis can be achieved in a number of ways, but the preferred pathway for the process of the invention is by controlled coprecipitation in alkaline medium of an aqueous mixture of ferrous (Fe^{2+}) and ferric (Fe^{3+}) salts. Ferrous ions can also be indicated as Fe (II), or iron (II), and ferric ions can be indicated by Fe (III), or iron (III). Although basic precipitation of ferric and ferrous salts is preferred for the process of the invention, other procedures for the preparation of magnetite can be adapted to the process of the invention. The overall equation for the preparation of

magnetite by base precipitation is:

$2\, Fe^{3+} + Fe^{2+} + 8\, (OH)^- \rightarrow Fe_3O_4 + 4\, H_2O$.

Water soluble salts of ferric (Fe^{3+}) and ferrous (Fe^{2+}) ions can be used with the process of the invention. For example, such water-soluble ferric and ferrous salts as halides, sulfates, acetates, and nitrates can be used. Preferred ferric (Fe^{3+}) and ferrous (Fe^{2+}) salts for the process of the invention are chlorides.

Aqueous solutions are defined as solutions which contain water. Aqueous solutions should contain sufficient water to dissolve the salts.

Preferred ratios of ferric to ferrous ions in the process of the invention are from about 1 to about 3. More preferred ratios of ferric to ferrous ions in the process of the invention are from about 1.5 to about 2.5. A most preferred ratio of ferric to ferrous ions in the process of the invention is about 2, so that stoichiometric amounts satisfying the above-mentioned reaction equation are provided.

The choice of the basic agent used to precipitate, or synthesize, magnetite within the microdroplets of the invention affects the structural and magnetic properties of the magnetite particles prepared. KOH, NaOH, LiOH are preferred bases for precipitating magnetite. A more preferred base is NH_4OH. An even more preferred base is TMAOH (tetramethylammonium hydroxide). Other tetraalkylammonium hydroxides can also be used.

Preferably a controlled excess of hydroxide is used to precipitate the magnetite. The pH of the aqueous solution within the microdroplets should be in the range of about 8 to 10. An aqueous magnetic colloid suspension can be obtained by coating the magnetite particles with a surfactant or a dispersing agent. Quaternary ammonium bases can be used to coat magnetite and stabilize it in colloidal suspension. The more preferred quaternary ammonium bases for stabilizing magnetite in solution belong to the class of quaternary ammonium hydroxides.

Low polarizing cations such as tetramethylammonium $N(CH_3)_4^+$ favor solution stability and give rise to well peptized solutions of magnetite. Coating magnetite particles with TMAOH as the surface active agent was found to be a very good way to achieve stable colloidal suspensions of magnetite. Therefore, a TMAOH coated magnetite particle comprises the preferred core for the polymeric microspheres prepared by the process of the invention.

Although preferred dispersing agents for the process of the invention belong to the classes of quaternary ammonium bases, other anionic, cationic, and non-ionic dispersing agents can be used with the process of the invention. For example, the dispersing agent can be chosen from organic or nonorganic chemicals which form low polarizing counterions in aqueous magnetite suspension. The preferred inorganic dispersing agents are nitric acid and perchloric acid. The preferred organic dispersing agents are tetraalkyl ammonium bases such as TMAOH. Other dispersing agents that can be used with the process of the invention include such water soluble polymers as polyvinylpyrrolidone, polyethylene glycol, copolymers of polyethylene glycol and polyalkylethylene glycol, polyacrylamide, and polyalkylacrylamide.

TMAOH has the additional feature of being a hydroxyl base with the capacity for precipitating magnetite from ferric and ferrous ions. Its dual role of both precipitating and stabilizing magnetite at one time could be partially responsible for the stable superparamagnetic magnetite particles prepared by the process of the method of the invention. TMAOH, in this dual role, is a preferred dispersing agent and base.

A colloidal suspension of magnetite stabilized with dispersing agent can be described as "stabilized" magnetite. Stabilized magnetite does not aggregate.

Although magnetite (Fe_3O_4) is a preferred superparamagnetic particle prepared by the process of the invention, other uniform-sized particles of single metal oxides, or mixtures of metal oxides, can also be prepared. Such particles include, but are not limited to, oxides of cobalt, chromium, molybdenum, manganese, nickel, vanadium, tungsten, zinc, iron and copper. The process of the invention can also be used to prepare polymeric microspheres that encapsulate inorganic particles other than magnetite, such as siliconoxide and other metal particles. Small metal particles are useful as catalysts in aqueous media.

The preferred embodiment of the process of the invention is carried out by dispersing an aqueous solution of ferric and ferrous salts into an AOT-toluene solution that contains monomer, initiator and preferably crosslinker. To this solution base and dispersing agent are added to precipitate magnetite and to stabilize it in the microemulsion. Preferably, a dispersing agent that is also a base is used in the magnetite precipitation step such that precipitation and stabilization occur in concert. Interaction of iron salts with base precipitates the magnetite. Preferably the interaction is carried out by using a controlled process.

Sonication using an ultrasonic probe is one method to mix the base with the iron salts.

The process of the invention is preferably carried out under deoxygenating conditions which can be achieved by bubbling inert gas through all reaction media and solutions. The removal of oxygen is more preferably carried out by bubbling nitrogen through the reaction media and solutions for at least 10 minutes, and even more preferably for at least 45 minutes. The nitrogen is preferably purified nitrogen. Deoxygenation is important both to prevent oxidation of Fe (II) before magnetite precipitation and magnetite after the synthesis. Deoxygenation is also important for carrying out the polymerization reaction.

In a preferred embodiment of the process of the invention, single superparamagnetic magnetite particles are encapsulated by a biocompatible homopolymer or co-polymer. "Polymer monomers" are the monomers that are polymerized in the process of the invention to form the polymeric coating of the stabilized magnetite particle. Preferred "polymer monomers" are methacrylic acid (MA) and 2-hydroxyethylmethacrylate (HEMA). These monomers, when polymerized, form a biocompatible random co-polymer shell around the magnetite particle. A preferred embodiment includes the ratio of methacrylic acid to hydroxy ethyl methacrylate from 100% methacrylic acid to about 0.1% methacrylic acid.

The hydroxyl groups of the 2-hydroxyethylmethacrylate can be activated by cyanogen bromide for the covalent bonding of proteins, and other chemicals containing amino groups, to the polymeric particles. Methacrylic acid residues, which carry the negative charge on the particles, prevent nonspecific binding to the cell surface. A variety of biochemical molecules can be attached to their carboxyl groups by the carbodiimide method.

Although methacrylic acid and hydroxyethyl methacrylate are preferred "polymer monomers" for the preparation of the polymer coating (hydrogel), numerous other "polymer monomers" could be used. Many monomers that form nontoxic vinyl polymers can be used. For example, the monomers that are suitable can be selected from amino, carboxyl or hydroxyl substituted acrylic monomers such as acrylamide, methacrylamide, acrylic acid, methacrylic acid, dimethylacrylamide or hydroxyl-lower alkyl or amino-lower alkylacrylates. It is noted that some monomers, e.g. acrylamide, are toxic while the corresponding polymers, e.g. polyacrylamide, are nontoxic. In such cases, monomers present after polymerization should be removed.

The process of the invention initiates the polymerization reaction by applying an

energy source to cause the initiation of the polymerization by decomposition of an initiator. Preferably this step is carried out by thermal decomposition of an initiator, through raising the temperature in the range from about 30°C to about 80°C. More preferably the temperature for initiating the polymerization is in the range of about 45°C to about 65°C. The invention can also use irradiation to carry out the polymerization. Suitable radiation sources for the process of the invention include ultrasonic radiation, microwave radiation, UV light, visible light, infrared light, gamma radiation, and X-ray radiation.

A "polymerization initiator" describes a chemical initiator that when activated initiates the polymerization reaction to form the polymer coat of the microsphere. The process of the invention can use either water-soluble or oil-soluble "polymerization initiators." Polymerization may be initiated by a free radical catalyst such as persulfate, peroxide, hydroperoxide or percarbonate. Other suitable initiators can be used. Preferred initiators are ammonium persulfate and potassium persulfate ($K_2S_2O_8$). A more preferred initiator is the hydrophobic initiator AIBN (2,2'-azobisisobutyronitrile). Examples of water soluble initiators are hydrogen peroxide, ammonium persulfate, with or without a reducing agent such as sodium bisulfite, sodium metabisulfite, sodium hydrosulfite, ferrous sulfate, and ammonium ferrous sulfate. Examples of oil soluble initiators are benzoyl peroxide, dicumyl peroxide, and azobisisobutyronitrile.

Selecting appropriate initiators based on their physical properties can be used as an additional parameter to control the size of the polymeric superparamagnetic microspheres.

In a preferred embodiment, polymer is cross-linked. The crosslinker used is called a "polymer crosslinker." Preferred crosslinkers are di- or poly acrylates such as N,N'-methylenebisacrylamide, ethylene glycol dimethacrylate, trimethylol-propane-trimethacrylate, and 1,3,5-triacryloyl-triazine. A more preferred crosslinker is pentaerythritol triacrylate. Preferably electrophoresis grade crosslinker is used.

After polymerization, the polymeric microspheres can be recovered by precipitation in an excess of organic solvents, such as an acetone-ethanol (9:1) mixture, followed by several washings, then centrifuged and dried under a vacuum. The solvents should be miscible with water but should not be solvents for the polymeric shell.

Water for preparation of solutions is preferably deionized, doubly distilled and deoxygenated prior to use.

Microemulsions have a dynamic structure wherein the droplets of the dispersed phase are diffusing through the continuous phase and colliding with each other. These droplets coalesce, temporarily merge and subsequently break to form separate droplets. This characteristic of microemulsions provides an additional means to control reactions by separating reactants in different microemulsion populations and then bringing the different microemulsions together to allow reactions to take place. Accordingly, the process of the invention can be carried out in a variety of different ways depending on how the reacting components are to be handled.

The formation of stable magnetite cores requires that a base and a dispersing agent be brought into contact with the ferrous and ferric ions. The invention can be carried out either by mixing two water-in-oil microemulsions or by adding a soluble base and dispersing agent directly to a water-in-oil microemulsion containing ferric and ferrous ions within the microdroplets. In the two water-in-oil microemulsion process, ferric and ferrous ions are contained within the microdroplets of a first microemulsion, and a second microemulsion containing base and water soluble dispersing agent within its microdroplets is added. Because the microdroplets collide and exchange contents, magnetite is formed and stabilized. Both of these processes can be used for precipitating ultrafine magnetite particles in a microemulsion droplet. In both processes, the contact of the reactants leads to the nucleation of the desired particles within the droplets.

The following examples are provided to assist in a further understanding of the invention. The particular materials and conditions employed are intended to be further illustrative of the invention and are not limiting upon the reasonable scope thereof.

EXAMPLES

Materials

Ferric chloride, ferrous chloride and tetramethylammonium hydroxide were furnished by the Aldrich Chemical Company, Inc. (Milwaukee, WI), and were used as received. AOT (sodium bis-2-ethyl-hexylsulfosuccinate), also called Aerosol OT, was purchased from Fluka Chemical Corp. (Ronkonkoma, NY) and purified as described in Schwertmann, U. and Cornell, R.M. *Iron Oxides in the Laboratory, Preparation and Characterization*, VCH Publishers, Inc., NY Chapter 11, pp. 11-114 (1991), which is herein incorporated by reference. Toluene was purchased from Aldrich (spectroscopy grade) and was used as

supplied. Tetramethylammonium hydroxide, in a water solution, was purchased from Fluka and used as supplied. Water was deionized, doubly distilled and deoxygenated prior to use. Methacrylic acid (Aldrich) was distilled at 40 °C (10 mm Hg) in the presence of copper shavings to remove inhibitor before use. Hydroxyethyl methacrylate (Sigma Chemical Company, St. Louis, MO) was distilled at 97°C (10 mm Hg) in the presence of copper shavings to remove inhibitors before use. Hydrophobic initiator, AIBN (2,2'-azobisisobutyronitrile) was purchased from Aldrich, recrystallized in ethanol and dried under vacuum. Hydrophilic initiator, potassium persulfate ($K_2S_2O_8$) was purchased from Fisher Scientific (Pittsburgh, PA) and used as supplied. Ammonium persulfate was purchased from Aldrich and used as supplied. Crosslinker N,N'-methylenebisacrylamide (electrophoresis grade) was purchased from Polysciences Inc. (Warrington, PA) and used as received.

The process of the invention can be carried out either by combining two preliminary microemulsions or preparing a single microemulsion.

EXAMPLE 1: Two-Microemulsion Process

In the two-microemulsion process, two preliminary microemulsions were prepared separately and then combined to form the reaction microemulsion.

The first preliminary microemulsion was prepared by dispersing a deoxygenated aqueous iron salts solution in a deoxygenated solution of AOT in toluene. The deoxygenated aqueous iron salts solution was prepared by using 0.1 grams (5.0×10^{-4} moles) of ferrous chloride and 0.16 grams (1×10^{-3} moles) of ferric chloride in 3 mL (0.17 moles) of deoxygenated, deionized, and doubly distilled water. The solution of iron salts (3 mL) was dispersed in a deoxygenated toluene solution of AOT, that was prepared by using 50 mL of toluene (0.47 moles) and 25 grams of AOT (5.6×10^{-2} moles).

A second preliminary microemulsion was prepared by dispersing 3 mL of a 12% deoxygenated tetramethylammonium hydroxide (TMAOH) solution (4.3×10^{-3} moles) in a deoxygenated toluene solution of AOT, that was prepared by using 25 grams of AOT (5.6×10^{-2} moles) and 50 mL of toluene (0.47 moles).

The microemulsions were mixed by sonication using an ultrasonic processor under nitrogen. The mixture of microemulsions turned intense black, indicating that magnetite was formed. The final microemulsion was stirred for an additional 20 minutes under nitrogen.

A solution of sodium methacrylate (1.57 grams, 1.45×10^{-2} moles),

hydroxyethylmethacrylate (0.07 gram, 5.38×10^{-4} moles), N,N'-methylenebiasacrylamide (0.0125 gram, 8.12×10^{-5} moles), and ammonium persulfate (0.023 grams, 1.0×10^{-4} moles) in 4 mL of deoxygenated, deionized, and doubly distilled water was added to the magnetite containing microemulsion. The molar ratio of water/AOT was 5/1. The polymerization was carried out at 55°C for 3 hours under nitrogen gas flow in order to insure the absence of oxygen. The polymeric microsphere (particles) were recovered by precipitation in an excess of an acetone/ethanol (9/1) mixture, followed by several washings using the same mixture. The polymeric microspheres were then centrifuged and dried under vacuum. Polymeric microspheres were resuspended in water and the average hydrodynamic radius of the particles, measured by the dynamic light scattering technique, was 91 nm. For the light scattering technique, see B. Chu, *Laser Light Scattering, Basic Principles and Practice*, Academic Press, Inc. (1991), which is herein incorporated by reference.

EXAMPLE 2: Single-Microemulsion Process

The single-microemulsion process was carried out as follows. The microemulsion was prepared by dispersing a deoxygenated aqueous solution of iron salts in a deoxygenated solution of AOT in toluene. The deoxygenated aqueous iron salts solution was prepared by using 0.15 grams (7.5×10^{-4} moles) of ferrous chloride and 0.24 grams (1.5×10^{-3} moles) of ferric chloride in 3 mL (0.17 moles) of deoxygenated, deionized, and double distilled water. The solution of iron salts (3 mL) was dispersed in a deoxygenated toluene solution of AOT, that was prepared by using 50 mL of toluene (0.47 moles) and 25 grams of AOT (5.6×10^{-2} moles). The microemulsion and 4.5 mL of a 12% (6.0×10^{-3} moles) solution of deoxygenated tetramethylammonium hydroxide (TMAOH) were mixed by ultrasonic processor under nitrogen gas flow. The molar ratio of water/AOT was 7.5/1. This represents one method of introducing base to the iron salts solution in the microdroplets. It is important that base enters the microdroplets at a slow and uniform rate. The intense black microemulsion was stirred for 20 minutes under nitrogen.

Methacrylic acid (1.25 grams, 1.45×10^{-2} moles), hydroxyethylmethacrylate (0.07 grams, 5.38×10^{-4} moles), N,N'-methylenebisacrylamide (0.0125 grams, 8.12×10^{-5} moles), and 2,2'- azobisisobutyronitrile (0.125 grams, 7.62×10^{-4} moles) were added to the magnetite containing microemulsion. The polymerization was carried out at 55°C for 3 hours under

nitrogen gas flow. The polymeric microspheres (particles) were recovered by precipitation in an excess of an acetone/ethanol (9/1) mixture, followed by several washings using the same mixture and then centrifugation. The polymeric microspheres were then dried under vacuum. The polymeric microspheres were resuspended in water, and the average hydrodynamic radius, measured by the dynamic light scattering technique, was 165 nm.

EXAMPLE 3: Single-Microemulsion Process

The magnetic polymeric microspheres were prepared as described in EXAMPLE 2 except that a solution of methacrylic acid (1.25 grams, 1.45×10^{-2} moles), hydroxyethylmethacrylate (0.07 grams, 5.38×10^{-4} moles), N,N'-methylenebisacrylamide (0.0125 grams, 8.12×10^{-5} moles), and 2,2'- azobisisobutyronitrile (0.125 grams, 7.62×10^{-4} moles) dissolved in 5 mL of toluene was added to the magnetite containing microemulsion. The molar ratio of water/AOT was 7.5/1. The average hydrodynamic radius of the polymeric microspheres (i.e., particles), measured by the dynamic light scattering technique, was 202 nm.

EXAMPLE 4: Single-Microemulsion Process

The magnetic polymeric microspheres were prepared as described in EXAMPLE 2 except that the volume of the tetramethylammonium hydroxide solution used was 7 mL. The molar ratio of water/AOT was 10/1. The average hydrodynamic radius of the polymeric microspheres, measured by the dynamic light scattering technique, was 208 nm.

EXAMPLE 5: Single-Microemulsion Process

The magnetic polymeric microspheres were prepared as described in EXAMPLE 4 except that the initiator was ammonium persulfate (0.023 grams, 1×10^{-4} moles) and the volume of the tetramethylammonium hydroxide solution was 6 mL. The molar ratio of water/AOT was 9/1. The average hydrodynamic radius of the particles (microspheres), measured by the dynamic light scattering technique, was 220 nm.

EXAMPLE 6: Single-Microemulsion Process

The single-microemulsion process was carried out as follows. The microemulsion

was prepared by dispersing a deoxygenated aqueous iron salts solution in a deoxygenated solution of AOT in toluene. The deoxygenated aqueous iron salts solution was prepared by using 0.15 grams (7.5×10^{-4} moles) of ferrous chloride and 0.24 grams (1.5×10^{-3} moles) of ferric chloride in 3 mL (0.17 moles) of deoxygenated, deionized, and double distilled water. The solution of iron salts (3 mL) was dispersed in a deoxygenated toluene solution of AOT, that was prepared by using 50 mL of toluene (0.47 moles) and 25 grams of AOT (5.6×10^{-2} moles). The microemulsion and 2.2 mL of a 25% solution of deoxygenated tetramethylammonium hydroxide solution were mixed by ultrasonic processor under nitrogen gas flow. The molar ratio of water/AOT was 7.5/1. This represents one method of introducing base to the iron salt solution in the microdroplets. It is important that base enters the microdroplets at a slow and uniform rate. The intense black microemulsion was stirred for 20 minutes under nitrogen.

Methacrylic acid (1.25 grams, 1.45×10^{-2} moles), hydroxyethylmethacrylate (0.07 grams, 5.38×10^{-4} moles), N,N'-methylenebisacrylamide (0.0125 grams, 8.12×10^{-5} moles), and an aqueous solution of ammonium persulfate (0.023 grams, 1.0×10^{-4} moles in 2.3 mL of water) were added to the magnetite containing microemulsion. The polymerization was carried out at 55°C for 3 hours under nitrogen gas flow. The polymeric microspheres (particles) were recovered by precipitation in an excess of an acetone/ethanol (9/1) mixture, followed by several washings using the same mixture and then centrifugation. The polymeric microspheres were then dried under vacuum. Polymeric microspheres were resuspended in water and the average hydrodynamic radius of the particles, measured by the dynamic light scattering technique, was 165 nm.

EXAMPLE 7: Single-Microemulsion Process

The magnetic polymeric microspheres (i.e., particles) were prepared as described in EXAMPLE 6 except that double the amounts of methacrylic acid (2.5 grams, 2.9×10^{-2} moles) and hydroxyethylmethacrylate (0.14 gram, 1.04×10^{-4} moles) were used and the crosslinker was pentaerythritol triacrylate (0.6 gram, 2×10^{-4} moles). The molar ratio of water/AOT was 7.5/1. The average hydrodynamic radius of the particles, measured by the dynamic light scattering technique, was 270 nm.

EXAMPLE 8: Single-Microemulsion Process

A microemulsion was prepared by dispersing a deoxygenated aqueous iron salts solution in a deoxygenated solution of AOT in toluene. The deoxygenated aqueous iron salts solution was prepared by using 0.15 grams (7.5×10^{-4} moles) of ferrous chloride and 0.24 grams (1.5×10^{-3} moles) of ferric chloride in 3 mL (0.17 moles) of deoxygenated, deionized, and doubly distilled water. A solution of sodium methacrylate (3.25 grams, 3×10^{-2} moles), pentaerythritol triacrylate (0.6 grams, 2×10^{-2} moles), and ammonium persulfate (0.023 grams, 1×10^{-4} moles) in 4.5 mL (0.25 moles) of deoxygenated, deionized, and doubly distilled water was prepared. These solutions were mixed together and then dispersed in a deoxygenated solution of AOT in toluene that was prepared by dissolving 25 grams of AOT (5.6×10^{-2} moles) in 50 mL of toluene (0.47 moles). The microemulsion and 2.2 mL of a 25% solution (6.0×10^{-3} moles) of deoxygenated tetramethylammonium hydroxide were mixed by ultrasonic processor under nitrogen gas flow. The intense black microemulsion was stirred for 20 minutes under nitrogen.

Before polymerization was started, 0.5mL of a 37% hydrochloric acid solution was added to the microemulsion. The molar ratio of water/AOT was 10/1. The polymerization was carried out at 55°C for 3 hours under nitrogen gas flow. The polymeric microspheres were recovered by precipitation in an excess of an acetone/ethanol (9/1) mixture, followed by several washings using the same mixture and then centrifugation. The polymeric microspheres were than dried under vacuum. The average hydrodynamic radius of the particles, measured by the dynamic light scattering technique, was 356 nm.

EXAMPLE 9: Magnetite Particle Preparation

The iron salts solution was prepared by using 0.15 grams (7.5×10^{-4} moles) of ferrous chloride and 0.24 grams (1.5×10^{-3} moles) of ferric chloride in 3 mL (0.17 moles) of deoxygenated, deionized, and doubly distilled water. The deoxygenated aqueous solution of iron salts (3 mL) was dispersed in a deoxygenated toluene solution of AOT, that was prepared by using 50 mL of toluene (0.47 moles) and 25 grams of AOT (5.6×10^{-2} moles). The microemulsion, and 2 mL of a 28% (6.1×10^{-3} moles) deoxygenated tetramethylammonium hydroxide solution, were mixed by using an ultrasonic processor under nitrogen gas flow. The intense black microemulsion was stirred for 20 minutes under nitrogen. The magnetite

particles were recovered by precipitation in an excess of an acetone/ethanol (9/1) mixture, followed by several washings using the same mixture and then centrifugation. The particles were then dried under vacuum. The magnetite particles were resuspended in water and the average hydrodynamic radius of the magnetite particles, measured by the dynamic light scattering technique, was 7.3 nm. The variance in size distribution of the magnetite particles was determined to be 0.2 from the light scattering measurements. These magnetite particles are the cores of the polymeric microspheres prepared by the process of the invention.

EXAMPLE 10: Superparamagnetic Magnetite Core

To assess the magnetic properties of the microspheres, vibrating-sample magnetometer (VSM) measurements were performed on the dried polymeric particles containing a magnetite core that were synthesized as described in Example 5. The curve of magnetization versus the applied magnetic field demonstrates that the magnetite is superparamagnetic. This curve is shown in Figure 1. The saturation magnetization was found to be equal to 2.72 emu/g which represents a magnetic content of 3.3%. This was calculated based on a value for pure magnetite of 81 emu/g.

The magnetization of the polymer coated particles remained the same for at least a month. This demonstrated that the magnetite core of the polymeric microspheres was stable against oxidation.

EXAMPLE 11: Atomic Force Microscopy

Atomic force microscopy (AFM) was used to demonstrate that the polymeric microspheres prepared by the process of the invention had a single magnetite core. The sample was prepared by dipping a silica plate in an aqueous suspension of magnetite containing polymeric microspheres that were synthesized as described in EXAMPLE 1. The microspheres were bonded to the plate. The phase mode of AFM is related to the deflection of the cantilever induced by the differences in elasticity in the particles studied. Therefore, an elastic surface, like the surface of the polymeric shell, will have a different response than the inelastic surface of the iron oxide core. Based on these differences, the internal morphology of the microspheres can be investigated. In the AFM picture as shown in Figure 2, the iron oxide core can be visualized as a single sharp circle surrounded by the shadow of the

polymeric shell. This demonstrates that the core is a single magnetite domain.

Thus, while there have been described what are presently believed to be the preferred embodiments of the present invention, those skilled in the art will realize that other and further embodiments can be made without departing from the spirit of the invention, and it is intended to include all such further modifications and changes as come within the true scope of the claims set forth herein.

WHAT IS CLAIMED IS:

1. A process for preparing superparamagnetic polymeric microspheres, comprising:

(a) forming a water-in-oil microemulsion which comprises a dispersion of an aqueous solution of salts of Fe (II) and Fe (III), polymerization initiator, polymer monomers, surfactant that locates at the interface of oil and water, and oil;

(b) adding base and a dispersing agent which is water soluble;

(c) mixing to precipitate core particles of magnetite within microemulsion microdroplets; and

(d) applying an energy source to the microemulsion with magnetite particles to cause polymerization of the monomers around the core particles, said polymerization taking place within the microemulsion microdroplets, thus forming uniform size microspheres containing a magnetite core with a polymer coating.

2. A process according to Claim 1, wherein the base and dispersing agent are added as an aqueous solution or as a microemulsion.

3. A process according to Claim 1, wherein the water-in-oil microemulsion further comprises polymer crosslinker.

4. A process according to Claim 1, wherein the surfactant is AOT (sodium-bis-2-ethyl-hexyl sulfosuccinate).

5. A process according to Claim 1, wherein the oil is selected from the group consisting of toluene and cyclohexane.

6. A process according to Claim 1, wherein the dispersing agent is tetramethylammonium hydroxide.

7. A process according to Claim 1, wherein the base is selected from the group

consisting of KOH, NaOH, LiOH and NH_4OH.

8. A process according to Claim 1, wherein both the dispersing agent and the base are tetramethylammonium hydroxide (TMAOH).

9. A process according to Claim 1, further comprising maintaining the microemulsions and solutions under deoxygenating conditions.

10. A process according to Claim 1, wherein the energy source comprises heat of at least 30°C.

11. A process according to Claim 1, wherein the mixing is carried out by sonication.

12. A process according to Claim 1, wherein the stoichiometric molar ratio of Fe (III) to Fe (II) is in the range from about 1 to about 3.

13. A process according to Claim 1, wherein the Fe salts are selected from the group consisting of halides, acetates and sulfates.

14. A process according to Claim 14, wherein the Fe salts are chlorides.

15. A process according to Claim 4, wherein the microemulsion comprises an AOT/toluene/water microemulsion which comprises AOT in an amount from about 10 weight percent to about 30 weight percent, water in an amount from about 2 weight percent to about 10 weight percent, and toluene in an amount from about 70 weight percent to about 80 weight percent based on 100 % AOT/toluene/water mixture.

16. A process according to Claim 15, wherein the mole ratio of water to AOT is from about 2 to about 20.

17. A process according to Claim 16, wherein the mole ratio of water to AOT is from about 5 to about 11.

18. A process according to Claim 1, wherein the initiator is hydrophobic.

19. A process according to Claim 1, wherein the initiator is selected from the group consisting of AIBN (2,2'-azobisisobutyronitrile), ammonium persulfate, and $K_2S_2O_8$ (potassium persulfate).

20. A process according to Claim 3, wherein the crosslinker is selected from the group consisting of N,N'-methylenebisacrylamide, and pentaerythritol triacrylate.

21. A process according to Claim 8, wherein TMAOH is a solution of about 1 weight percent to about 25 weight percent.

22. A process according to Claim 1, wherein the polymer monomers are selected from the group consisting of methacrylic acid and hydroxyethyl methacrylate.

23. A process according to Claim 22, wherein the ratio of methacrylic acid to hydroxyethyl methacrylate is from about 100 to about 0.1.

24. Polymeric microspheres produced according to the process of Claim 1.

25. Polymeric microspheres comprising a core which comprises a single superparamagnetic magnetite domain and a polymer coating (hydrogel) around the core.

26. The polymeric microspheres of Claim 25, wherein the core is stabilized magnetite.

27. The polymeric microspheres of Claim 25, wherein within the microspheres, as synthesized, the variance of the core size distribution is about 0.2.

28. The polymeric microspheres of Claim 25, wherein the core has a radius from about 3 nm to about 20 nm.

29. The polymeric microspheres of Claim 28, wherein the core has a mean radius from about 7.2 nm to about 7.4 nm.

30. The polymeric microspheres of Claim 25, wherein the polymeric coating is a vinyl polymer.

31. The polymeric microspheres of Claim 30, wherein the polymeric coating is a random copolymer comprising monomers selected from the group consisting of methacrylic acid and hydroxyethyl methacrylate.

32. The polymeric microspheres of Claim 31, wherein the polymeric coating is a random copolymer containing from about 5 % to about 20 % hydroxyethyl methacrylate.

33. The polymeric microspheres of Claim 25, wherein the polymeric microspheres have a polymeric coating (hydrogel) which is substantially uniform in thickness among the microspheres, said thickness from about 10 nm to about 400 nm.

34. The polymeric microspheres of Claim 25, wherein the variance of size distribution of the polymeric microspheres is about 0.02.

35. The polymeric microspheres of Claim 25, wherein the magnetite is about 3.3 weight percent of each microsphere.

36. The polymeric microspheres of Claim 25, wherein the saturation magnetization of the magnetite is about 2.72 emu/g.

37. The polymeric microspheres of Claim 25, wherein the magnetite core is stable against oxidation in aqueous solutions.

AMENDED CLAIMS

[received by the International Bureau on 12 March 1999 (12.03.99); original claim 14 replaced by amended claim 14, remaining claims unchanged (1page)]

consisting of KOH, NaOH, LiOH and NH_4OH.

8. A process according to Claim 1, wherein both the dispersing agent and the base are tetramethylammonium hydroxide (TMAOH).

9. A process according to Claim 1, further comprising maintaining the microemulsions and solutions under deoxygenating conditions.

10. A process according to Claim 1, wherein the energy source comprises heat of at least 30°C.

11. A process according to Claim 1, wherein the mixing is carried out by sonication.

12. A process according to Claim 1, wherein the stoichiometric molar ratio of Fe (III) to Fe (II) is in the range from about 1 to about 3.

13. A process according to Claim 1, wherein the Fe salts are selected from the group consisting of halides, acetates and sulfates.

14. A process according to Claim 1, wherein the Fe salts are chlorides.

15. A process according to Claim 4, wherein the microemulsion comprises an AOT/toluene/water microemulsion which comprises AOT in an amount from about 10 weight percent to about 30 weight percent, water in an amount from about 2 weight percent to about 10 weight percent, and toluene in an amount from about 70 weight percent to about 80 weight percent based on 100 % AOT/toluene/water mixture.

16. A process according to Claim 15, wherein the mole ratio of water to AOT is from about 2 to about 20.

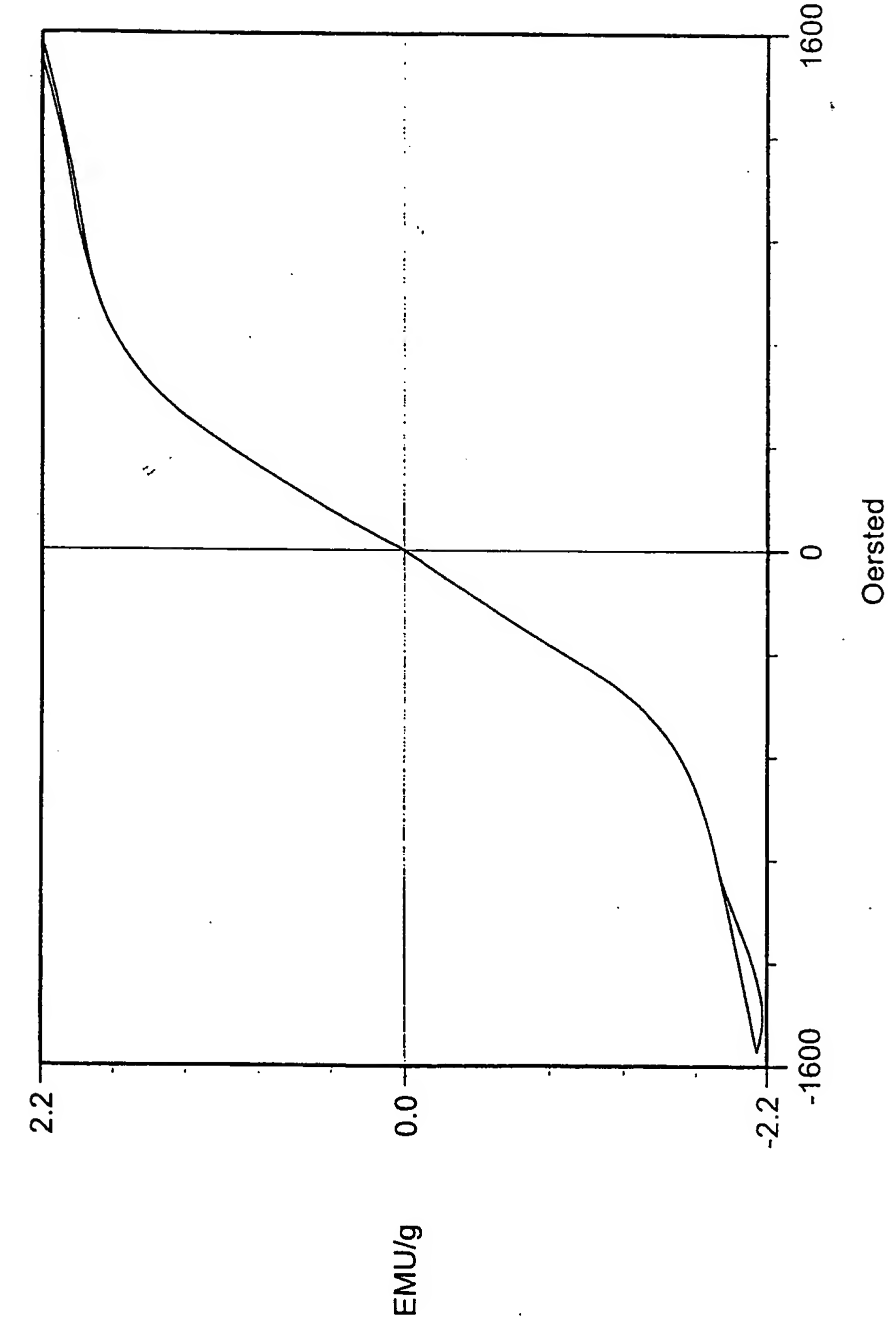
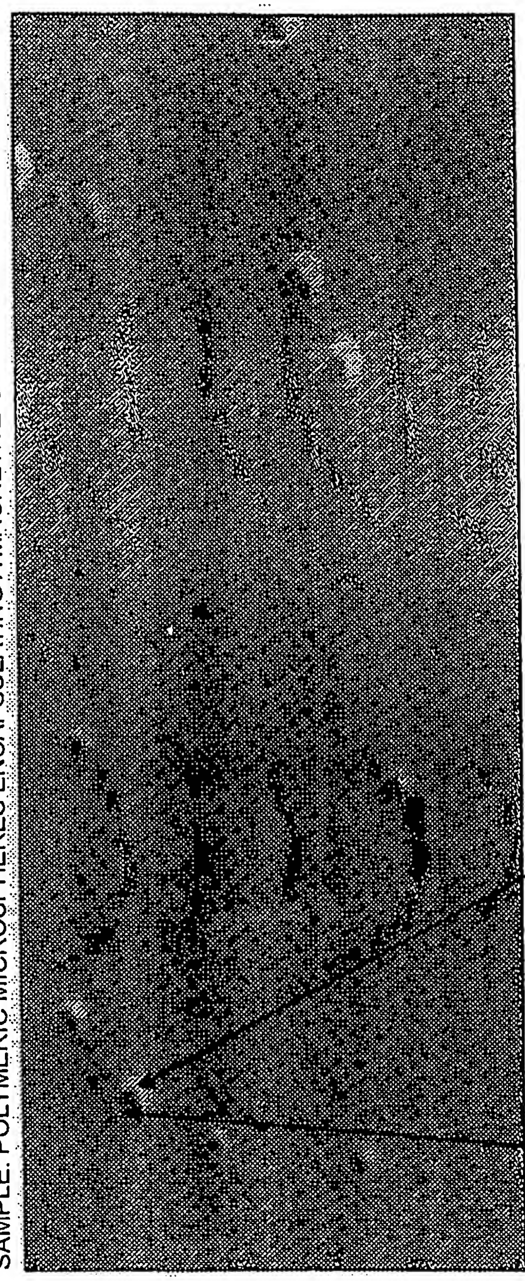


FIG-1

FIG-2


SAMPLE: POLYMERIC MICROSPHERES ENCAPSULATING A MAGNETITE CORE
PHASE MODE
POLYMERIC SHELL
MAGNETITE CORE
CORE-SHELL STRUCTURE CONFIRMED

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/21266

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 49/00; A61B 5/05; G01N 33/553; C08K 9/10
US CL :424/9.322, 9.323; 523/201, 202, 223; 524/801, 832
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/9.322, 9.323; 523/201, 202, 223; 524/801, 832

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS; superparamagnetic, microsphere#, coat##, emulsion, polymerization, magnetite, hydroxyethyl methacrylate

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CANDAU F. et al. Inverse Microemulsion Polymerization of Acrylamide: Characterization of the Water-in-Oil Microemulsions and the Final Microlatexes, J. of Colloid and Interface Science, Vol. 101 September 1984, pages 167-183, especially abstract.	1-13, 15-24
Y	LIZ, L. et al. Preparation of colloidal Fe_3O_4 ultrafine particles in microemulsions, J. of Material Science, 1994, Vol. 29, pages 3797-3801, especially page 3797.	1-13, 15-24
X ----- Y	US 5,597,531 A (LIBERTI et al) 28 January 1997 (28-01-97), table 1, column 4, lines 43-65 and column 6, lines 11-18.	25, 26, 30, 36 and 37 ----- 25-37

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 DECEMBER 1998	20 JAN 1999

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	TAE H. YOON
Facsimile No. (703) 305-3230	Telephone No. 703-308-0661

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	US 5,219,554 A (GROMAN et al) 15 June 1993 (15-06-93), column 5, lines 3-11, column 7, lines 49-61, column 10, lines 62-68 and column 16.	25, 26, 28, 36, 37 ----- 25-37
X ----- Y	US 4,965,007 A (YUDELSON) 23 October 1990 (23-10-90), column 4, lines 50-58, column 7, lines 25-52 and column 6, lines 46-55,	25-30, 33-37 ----- 25-37
X ---- Y	FRANK S. et al. Voltage-sensitive magnetic gels as magnetic resonance monitoring agents, Nature May 1993, Vol 363, pages 334-336, especially page 334.	25-28, 30, 34-37 ----- 25-37
Y	US 4,157,323 A (YEN et al) 05 June 1979 (05-06-79), abstract and column 6, lines 22-38.	25-37
X	US 5,492,814 A (WEISSLEDER) 20 February 1996 (20-02-96), column 5, lines 13-34, column 6, lines 27-52 and column 15, lines 4-44.	25-29, 33-37

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/21266

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 14
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

the claim is self-dependent.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.